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DRUG DISCOVERY

Exploring the anti-inflammatory potentials of *N*-((5-(((1,3-dioxoisoindolin-2-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide

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In the process of drug discovery, several non-steroidal anti-inflammatory drugs (NSAIDs) have been developed, however, a majority of them suffered from pharmacodynamics, pharmacokinetic, side-effects, or adverse drug reactions, which compelled researchers for continuous searching for better alternatives. The present research involved rational synthesis of *N*-((5-(((1,3-dioxoisoindolin-2-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide from the starting material *N*-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide (which in turn was formed by the reaction of hippuric acid with thiosemicarbazide in the presence of H_2SO_4) with phthalimide in the presence of formaldehyde, followed by exploration of *in vivo* anti-inflammatory potential by utilizing the carrageenan-induced paw edema method. The compound presented noteworthy activity as compared to that of standard drug indomethacin, probably by inhibiting the inflammatory mediators like COX-1/2 and LOX. The research will definitely open new avenues to the medicinal chemists for further development of anti-inflammatory drugs with pronounced activity along with a better safety profile.

INTRODUCTION

Inflammation is the most imperative process of the human body which acts as the first-line of defense against harmful pathogens (Mahapatra *et al*., 2018). It is often characterized by redness, warmth, swelling, and pain and sometimes immobility (Amdare *et al*., 2017). At the same time, the process of inflammation can also be problematic, though; it is known to play an imperative role in the pathogenesis of some chronic diseases (Mahapatra *et al*., 2018a). In the process of drug discovery, several non-steroidal anti-inflammatory drugs (NSAIDs) have been developed, however, the majority of them suffered from either pharmacodynamics, pharmacokinetic, side-effects, or adverse drug reactions (Mahapatra *et al*., 2018b), which compelled researchers for the continuous search for better alternatives (Mahapatra *et al*., 2017).

Thiadiazole is one of the privileged heterocycles in medicinal chemistry having multifarious pharmacological potentials such as anti-bacterial, anti-fungal, anti-cancer, anti-ulcer, anti-convulsant, anti-inflammatory, anti-tubercular, anti-viral, anti-leishmanial, anti-trypanosomal, anti-oxidant, etc (Hu *et al*., 2014). Recently, a number of hybrid scaffolds of 1,3,4-thiadiazole have been reported like 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole (Labanauskas *et al*., 2001), 5-(1-adamantyl)-1,3,4-thiadiazole (Kadi *et al*., 2010), 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles (Karegoudar *et al*., 2008), methylene bridged benzofuranyl imidazo[2,1-b][1,3,4]thiadiazoles (Jadhav *et al*., 2008),1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (Gilani *et al*., 2010), 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles (Mullican *et al*., 1993), 3-(2,4-dichlorophenoxy)methyl)-1,2,4-triazolo-thiadiazole (Shehry *et al*., 2010), spiro-xanthene-9′,2-[1,3,4]thiadiazole (Hafez *et al*., 2008), 2-Amino-5-sulfanyl-1,3,4-thiadiazole (Sainy *et al*., 2008), 2-trifluoromethyl/sulfonamido-5,6-diaryl substituted imidazo[2,1-b]-1,3,4-thiadiazole (Gadad *et al*., 2008), etc.

Phthalimide also finds importance in inflammation conditions as a potent inhibitor of inflammatory mediators. In the journey of drug discovery, alkyl-substituted phthalimide 1*H*-1, 2, 3-triazole derivatives (Assis *et al*., 2012), oxadiazolo-phthalimides (Antunes *et al*., 2003), mandelic acid derived phthalimides (Varala *et al*., 2008), arylphthalimides (Assis *et al*., 2014), etc. have been found to be potent anti-inflammatory candidates.

The present research involved rational synthesis of *N*-((5-(((1,3-dioxoisoindolin-2-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide from the starting material *N*-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide (which in turn was formed by the reaction of hippuric acid with thiosemicarbazide in the presence of H_2SO_4) with phthalimide in the presence of formaldehyde, followed by exploration of *in vivo* anti-inflammatory potential by utilizing the carrageenan-induced paw edema method.

MATERIALS AND METHODS

Chemicals and instrumentation

The starting material *N*-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide was obtained from our previous report. The

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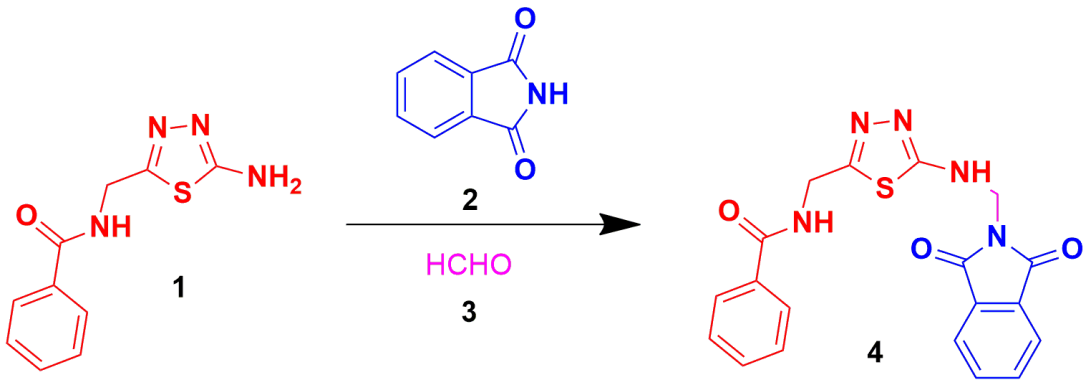

Scheme 1 Synthetic protocol for the hybrid 1,3,4-thiadiazole derivative

Table 1 *In vivo* anti-inflammatory activity of the hybrid 1,3,4-thiadiazole derivative

Group	Percentage (%) inhibition of edema		
	1 hr	2 hr	3 hr
3	31.71 ± 1.33	46.92 ± 1.49	64.19 ± 1.66
Indomethacin	45.63 ± 1.85	58.27 ± 2.02	78.51 ± 1.97

n = 6; ED_{50} of 200 mg/kg b.w. in male adult albino mice; P < 0.01

analytical grade chemical reactants and solvents were procured from Sigma Aldrich, Germany through a local vendor. The final product was analyzed using Fourier Transformed Infrared (FT-IR) Spectroscopy (Shimadzu® IRAffinity-1), Mass Spectroscopy (MICROMASS Q-TOF instrument), CHN analyses (PerkinElmer Elemental Analyzer 2400), and ^{1}H-NMR (Bruker Avance-II). The FT-IR data were expressed in cm^{-1} and proton-NMR in ppm. The chemical reaction progress was monitored by Merck® pre-coated silica gel-G TLC plates.

Animals

The anti-inflammatory potential of the compound was screened in albino rats of following attributes, after obtaining Department Ethical Committee permission and CPCSEA (1389/a/10/CPCSEA): 6 to 7 weeks age, 180-220 g body weight, kept in the animal house in polypropylene cage (two animals per cage) under the conditions of 25–26ºC temperature, 12 hr light and dark cycle, and humidity 50–65%. The animals were given standard rodent pellet feed and provided free access to the water.

Synthesis of target compounds

The present synthesis involves the chemical reaction of *N*-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide **(1)** with phthalimide **(2)** in the presence of formaldehyde **(3)** to yield the desired product **(4)** by following the Mannich reaction, one of the most important C-C bond-forming reactions. Here, the three components containing an active hydrogen atom was allowed to react with an NH-amine derivative and formaldehyde to form a condensed product. The crucial characteristic of the chemical reaction involved substitution of the active hydrogen atom by an aminomethyl group. The outline of the chemical reaction is given in **Scheme 1**.

Synthetic protocol for N-((5-(((1,3-dioxoisoindolin-2-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide **(4)**

A methanolic solution of *N*-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide **(1)** (0.01 M) was taken into a three-neck flask equipped with a stirrer and a dropping funnel. The solution was stirred at high RPM for the duration of 15-20 minutes to dissolve the content completely. Subsequently, the formaldehyde **(3)** (10 mL) was added dropwise to the solution for the period of 7-8 minutes. Then, the resultant mixture was stirred for 30 minutes at the temperature of 40±1°C to form methylol derivative. Afterward, phthalimide **(2)** (in the form of methanolic solution) was added to the above solution dropwise with constant stirring in the period of 30 minutes. The reaction mixture was refluxed for the duration of 3-4 hrs. The content was poured over crushed ice-water to filter off the solid product. The final product **(4)** was washed thoroughly with hot water, air dried, and recrystallized suitably.

68% yield; FTIR (KBr) υ (cm^{-1}): 3181 (-NH, stretching), 3128 (C-H, aromatic), 1727 (C=O, stretching), 1695 (C=N, five membered), 1644 (C=C, aromatic), 1607 (-NH, bending), 1452 (-CH_2, bending), 1288 (C-N, stretching); ^{1}H NMR (δ, ppm, $CDCl_3$): 8.26 (8, Amide, 1H), 7.3-8.1 (Aromatic, 9H), 5.37 (14, Methylene, 2H), 4.81 (9, Methylene, 2H), 4.42 (13, Amide, 1H). MS: M^+ 393. Anal. Calcd. for $C_{19}H_{15}N_5O_3S$: C, 58.01; H, 3.84; N, 17.80. Found: C, 57.22; H, 3.19; N, 17.14

Acute toxicity studies

The *in vivo* acute toxicity profile of the compound was determined to estimate the maximum safe dose at which no toxic symptoms will be seen. It was found out by escalating the dose in the range of 25-500 mg/kg. The safety of the compound was calculated based on the LD_{50} value (Kanhed *et al*., 2016).

Anti-inflammatory screening

The *in vivo* anti-inflammatory activity of the compound was estimated using carrageenan-induced paw edema method. Before the commencement of the protocol, the albino rats have fasted overnight and distilled water (5 mL) was administered orally to all albino rats. Afterward, the animals received 1% carrageenan solution at the right hind paw (at the subplanter region) by using an injection. Subsequently, an hour before the study, the rats received compound (200 mg/kg b.w.) by suspending in 0.9% saline solution. The rats in the control group were administered in 0.9% saline solution (containing Tween 80). The paw thickness was estimated using a digital mercury micrometer for 3 hrs duration at an interval of 1 hr. The reduction in edema was computed by the variation in the width of the injected and non-injected paws (Mahapatra *et al*., 2017a).

Statistical treatment

The procured data were analyzed statistically by using the one-way ANOVA method followed by Dunnett's multiple comparison test. P value of <0.01 was considered significant.

RESULTS AND DISCUSSION

Chemistry

The spectroscopic techniques assisted in deriving adequate proof for the conversion of starting material into the desired product. The formation of the hybrid 1,3,4-thiadiazole derivative **(4)** was supported by the disappearance of the $–NH_2$ peak at FT-IR spectra of the starting material **(1)** at 3264 cm^{-1}. In addition to this, the presence of two amides at positions 8 and 13 was predominantly observed at 8.26 ppm and 4.42 ppm, respectively. Furthermore, the stretching and bending in the FT-IR spectra at 3181 cm^{-1} and 1607 cm^{-1} supported the formation of the desired pharmacophore. The methylene components were noticed both in the FT-IR and ^{1}H-NMR spectra. The components at 9 and 14 positions were detected at 5.37 ppm and 4.81 ppm, respectively. Moreover, the observed bending in the FT-IR spectra at 1452 presented the attachment of phthalimide portion with the thiadiazole scaffold. On scrutinizing the mass spectra, it was seen that the base peak of the hybrid compound corresponded closely with the calculated molecular mass (393). A number of fragment peaks of m/z 100-150 also appeared in the mass spectra. Besides, the CHN analysis of the compound represented % elemental compositions in close agreement with the theoretical values.

Acute toxicity studies

The compound displayed no acute toxic symptoms over the tested range of 25-500 mg/kg. The *in vivo* screening was performed at 200 mg/kg b.w.

Anti-inflammatory activity

The hybrid 1,3,4-thiadiazole derivative **(4)** expressed a noteworthy *in vivo* anti-inflammatory activity when screened through the carrageenan-induced paw edema method. The compound displayed comparable activity than that of the standard drug indomethacin, with % edema reduction of 31.71, 46.92, and 64.19, respectively, at the three consecutive hours. The compound may be believed to successfully inhibit the mediators of inflammation like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX).

CONCLUSION

The present research aimed at exploring the *in vivo* anti-inflammatory potential of *N*-((5-(((1,3-dioxoisoindolin-2-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide using carrageenan-induced paw edema method where the compound presented noteworthy activity as compared to that of standard drug indomethacin, probably by inhibiting the inflammatory mediators like COX-1/2 and LOX. The research will definitely open new avenues to the medicinal chemists for further development of anti-inflammatory drugs with pronounced activity along with a better safety profile.

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